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SPATIAL DISTRIBUTION AND PACKING OF XYLEM CONDUITS¹

JORDI MARTÍNEZ-VILALTA^{2,3,5}, MAURIZIO MENCUCCINI^{2,4}, XAVIER ÁLVAREZ³, JUAN CAMACHO³,
LASSE LOEPFE², AND JOSEP PIÑOL^{2,3}

²CREAF, Cerdanyola del Vallès 08193, Spain; ³Univ Autònoma Barcelona, Cerdanyola del Vallès 08193, Spain; and ⁴University of Edinburgh, School of GeoSciences, Crew Building, West Mains Road, EH9 3JN Edinburgh, UK

- *Premise of the study:* The hydraulic properties of the xylem determine the ability of plants to transport water from the soil to the leaves and to cope with important stress factors such as frost and drought. Hydraulic properties have usually been studied as a function of the anatomy of xylem conduits and their pits, but recent studies have proposed that system-level properties, related to the topology of the xylem network, may also play a role. Here we study how the spatial arrangement of conduits in xylem cross sections affects the relationship between mean conduit lumen area and conduit density (packing function) across species.
- *Methods:* Point pattern analysis was used to describe the spatial distribution of xylem conduits in 97 woody species. The effect of conduit aggregation on the packing function was tested using phylogenetic generalized least squares. A hydraulic model with an explicit description of the topology of the xylem network was used to interpret the functional significance of our findings.
- *Key results:* The spatial arrangement of conduits affected the packing function across species, so that species with aggregated distributions tended to have lower conduit densities for a given conduit size and lower conduit lumen fractions. According to our modeling results, the higher conduit-to-conduit connectivity of species with aggregated distributions allows them to achieve higher hydraulic conductivity. Species with aggregated conduits, however, pay a cost in terms of increased vulnerability to embolism.
- *Conclusions:* The spatial arrangement of conduits affects the fundamental structural and functional attributes of the xylem.

Key words: cavitation; embolism; hydraulic conductivity; network connectivity; packing; water transport; xylem anatomy.

The xylem of plants provides a low-resistance pathway for water transport between roots and leaves (Tyree and Zimmermann, 2002; Holbrook and Zwieniecki, 2005). Xylem properties are a key element to understand plant water use and plant responses to pervasive stress factors such as frost (e.g., Pockman and Sperry, 1996) and droughts (e.g., Brodribb and Cochard, 2009), ultimately controlling the distribution of vegetation and ecosystem function at a global scale (e.g., McDowell et al., 2011). Two main properties determine the performance of the xylem as a water-conducting system: its maximum capacity to transport water, measured in terms of hydraulic conductivity, and its vulnerability to embolism, usually characterized by the water potential causing a 50% decline in hydraulic conductivity (P_{50}). The xylem has also an important role in providing mechanical strength to the stem (e.g., Baas et al., 2004), but these biomechanical considerations are outside the scope of this paper.

Structurally, the xylem is a network of interconnected conduits. The hydraulic properties of the xylem network depend heavily upon wood anatomical properties such as conduit density and length and diameter distributions (Tyree et al., 1994; Comstock and Sperry, 2000), as well as on the characteristics of interconduit pits and pit-membranes (Choat et al., 2008). At the same time, however, tissue-level properties related to the

arrangement of xylem conduits in space also have the potential to affect xylem function. These tissue-level properties have been studied from different perspectives and under different names: vessel grouping (Carlquist, 1984; Lens et al., 2011), hydraulic segmentation (Tyree and Zimmermann, 2002), sectoriality (Zanne et al., 2006), redundancy (Ewers et al., 2007), conduit-to-conduit connectivity (Loepfe et al., 2007) and hydraulic integration (Schenk et al., 2008; Espino and Schenk, 2009).

Tissue-level effects, however, have proved difficult to quantify, mostly due to the inherent difficulties in describing the three-dimensional (3D) structure of the xylem network of real plants with the required level of detail (cf. Zimmermann and Tomlinson, 1966; Trtik et al., 2007; Brodersen et al., 2011 for attempts to apply different technologies). So far, the best empirical approaches to study tissue-level effects on xylem hydraulics have used cross sections and are therefore two-dimensional (2D). Long ago, Carlquist (1984) proposed an index of vessel grouping in cross-sections as a measure of conduit aggregation and hypothesized that vessel grouping is advantageous for dicotyledonous species living in dry habitats (cf. Carlquist, 2009 and references therein). More recently, Lens et al. (2011) provided the first direct empirical evidence showing that vessel grouping is correlated with both hydraulic conductivity and vulnerability to embolism. Using a more theoretical approach, Loepfe et al. (2007) used a hydraulic model including a full characterization of the topology of the xylem network to show that connectivity (i.e., the average number of conduit neighbors connected to a given conduit) increases both maximum hydraulic conductivity (by providing alternative pathways for water movement) and vulnerability to embolism (by facilitating the spread of emboli). However, a quantitative framework relating vessel grouping in 2D with network properties in 3D and xylem function is currently lacking.

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⁵Author for correspondence (e-mail: Jordi.Martinez.Vilalta@uab.es)

A key aspect of interspecific variation in xylem structure is the strong negative relationship between mean conduit lumen area (or diameter) and conduit density (Baas, 1973; Sperry et al., 2008) reflecting, at least in part, the fact that the space available for conduit lumens is bounded by the cross-sectional area of the xylem (the “packing limit”). Recently, Zanne et al. (2010) developed two indices (the conduit lumen fraction, F , and the size to number ratio, S) to encapsulate the functional implications of different combinations of conduit lumen area and density. In another recent paper, McCulloh et al. (2010) showed for the first time that the relationship between lumen area and density (i.e., the packing function) differs among functional wood types, a result they interpret in terms of conduit tapering and the scaling of hydraulic conductivity with stem diameter. One of the main differences among functional wood types is the spatial distribution of xylem conduits (cf. Mencuccini et al., 2010). However, the implications of the spatial arrangement of conduits on their packing have never been explored explicitly.

Here, we used the methodology recently developed by Mencuccini et al. (2010) to retrieve quantitative information on the spatial arrangement of xylem conduits from cross sections of 97 woody species. We then studied the relationship between the resulting spatial distributions and the observed packing of xylem conduits at the species level. Additionally, we used a development of the Loepfe et al. (2007) water transport model to (1) convert different spatial patterns of conduits in cross sections into full 3D network structures and (2) assess the implications of different spatial arrangements of xylem conduits in terms of maximum hydraulic conductivity and vulnerability to embolism. We hypothesized that species with more aggregated conduit distributions will obtain a benefit in terms of increased hydraulic conductivity and that these species will therefore have a packing function characterized by relatively low conduit densities for a given lumen area.

MATERIALS AND METHODS

Characterizing the spatial arrangement of xylem conduits—The methods are described in full in Mencuccini et al. (2010). Xylem anatomical cross sections were downloaded from Schoch et al. (2004). Of the cross-sectional images included in the database, those corresponding to 105 species were of sufficient quality to be used for further quantitative analysis. For each species, the best cross-sectional image was selected out of the 1–6 available. For all images, the analyzed area effectively spanned one growth ring selected among those present in the image. A semiautomated image analysis procedure was employed to determine the x and y coordinates of the centers of each conduit, as well as their respective lumen areas using standard shareware software (ImageJ v.1.40, available from <http://rsb.info.nih.gov/ij/>). Image-by-image checking and manual corrections were necessary to exclude nonvascular elements or include conduits that had not been selected.

Point pattern analysis (Bivand et al., 2008) was used to describe the spatial distribution of xylem conduits. We fitted non-Poisson models accounting for the presence of point-to-point interactions (“attraction” or “repulsion”) and extracted a quantitative index of conduit aggregation from those models. The model that we selected for modeling xylem cross sections was a piecewise Geyer model (Geyer, 1999). The second interaction parameter of this model (γ_2) was used to characterize the spatial distribution of conduits (cf. Mencuccini et al., 2010): $\gamma_2 < 1$ implies a uniform distribution, $\gamma_2 > 1$ describes aggregated distributions, and if $\gamma_2 = 1$ the model reduces to a random Poisson process. Please note that conduit “aggregation” and “grouping” are considered as synonyms throughout the manuscript. Our final data set comprises the 97 species for which a proper characterization of their conduit spatial patterns was possible (Appendix S1, see Supplemental Data with the online version of this article).

Data analysis—Analysis of variance was used to test for differences in xylem anatomical properties across wood types (categorical variable with four

levels: conifer, diffuse-porous, semi-ring-porous, ring-porous; cf. online Appendix S1). We used linear models to assess the effect of a set of explanatory variables on a given response variable. Both ordinary least squares (OLS) and major axis (MA) estimation were used to estimate the slope of the packing function. Phylogenetic effects were explicitly taken into account by using phylogenetic generalized least squares (PGLS; Paradis, 2006). Besides phylogenetic structure, our PGLS model of conduit density included the effects of lumen area, wood type, and conduit aggregation (parameter γ_2). Model selection started with the saturated model including second order interactions and continued by removing the least significant term until there was no further reduction in the Akaike information criterion (AIC). PGLS models were also used to study the relationship between the aggregation parameter (γ_2) and the conduit lumen fraction (F) and the size to number ratio (S), accounting for wood type. We defined F as the product of mean lumen area and conduit density, and S as the ratio between mean lumen area and conduit density, following Zanne et al. (2010). Although F and S both contribute to determine xylem hydraulic conductivity, the effect of F is proportionally larger (Zanne et al., 2010). To account for this, we also calculated $K = \sqrt{(S \cdot F^3)}$ as a crude index of specific hydraulic conductivity (cf. Zanne et al., 2010).

The final phylogenetic tree as well as further details on the phylogenetic analyses are given in Mencuccini et al. (2010). We fitted each PGLS model assuming a Brownian or an Ornstein–Uhlenbeck (OU) model of character evolution. Ornstein–Uhlenbeck models incorporate both selection and drift and are thus more general than pure drift models based on Brownian motion. Since the results for these two models were similar, and the OU model fitted the data slightly better, we only report the results obtained using the OU model. All analyses were carried out using the packages *smatr* and *nlme* of the R software (version 2.7, R Foundation for Statistical Computing). In all cases, variables were log-transformed when required to satisfy the normality assumptions or to improve the linearity of relationships, as explained in the text.

The hydraulic model—The hydraulic model was a modification of the model described in Loepfe et al. (2007). The model simulates an ideal xylem segment opened to the atmosphere at both ends. The xylem is modeled as a 3D set of conduits interconnected through porous membranes. The conduits are created from a homogeneous 3D lattice in which the longitudinal distance between two adjacent nodes represents a vessel element (or a tracheid), and each node has a given probability to become the end of a conduit, which leads to a negative exponential distribution of conduit lengths (Nijse, 2004). The proportion of nodes occupied by conduits in (virtual) cross sections is a function of conduit density, as introduced by the user. Each conduit is randomly assigned a diameter that determines its hydraulic resistance. The model implements two alternative assumptions regarding the relationship between the length and diameter of individual conduits. In the first one, conduit length and diameter are considered independent within a wood segment. In the second, an explicit link between conduit length and diameter is introduced using an allometric relationship (with a scaling exponent of 0.67, cf. Sperry et al., 2006).

Adjacent conduits (i.e., those separated by only one edge in a simulated cross section) are considered to effectively touch each other and are always connected by pit membranes. The resistance of these interconduit connections is added to the resistance of the lumens to get an overall conduit resistance. A linear system is obtained by assuming Darcy’s flow and steady state conditions. The system is solved numerically to obtain the equilibrium pressure at each node of the 3D grid. The conductivity of the xylem segment is defined as the overall flow divided by the (external) pressure gradient driving the flow. We applied a gradually increasing external positive air pressure (P) simulating the air-injection technique to establish vulnerability curves to drought-induced embolism (Cochard et al., 1992). By gradually increasing P , we obtained conductivities at different pressures and the corresponding vulnerability curve and P_{50} value (cf. Pammeter and Vander Willigen, 1998).

The main difference between the model used here and Loepfe et al. (2007) is the way in which the xylem structure was grown. Here we started from a cross section with a given density of conduits and a spatial distribution described by the parameters of a piecewise Geyer model (cf. above, see also Mencuccini et al., 2010). This structure was propagated in the axial direction by keeping constant both the density and the spatial distribution of conduits in the cross section, while allowing for some tortuosity in the conducting pathways along the axial direction. To do that, a parameter was introduced, Persistence, that determines the proportion of conduit ends from which a new conduit was created. Persistence = 1 implies that there is one new conduit starting exactly at (and connecting to) each conduit end and, therefore, that there is no tortuosity (i.e., all conducting paths are straight and parallel). If Persistence < 1, a proportion of conduits ending at a given level, equal to $1 - \text{Persistence}$, are not continued

by a new conduit located exactly on top of their final element. Instead, the number of new conduits required to keep conduit density constant are created at nonoccupied nodes using the same grouping algorithm as above. The closer Persistence is to zero the higher the overall tortuosity of the simulated xylem network. As in Loepefe et al. (2007), connectivity is defined as the average number of (different) conduits connected to a given conduit throughout its full length. A detailed description of the model can be found in Appendix S2 (see online Supplemental Data). The program code is written in C++ (Dev C++ 4.9.9.2) and is available upon request.

Simulation experiments—The model was run with a parameter set similar to that used in Loepefe et al. (2007) (Table 1). Conduit length and diameter distributions were assumed to be independent in the initial set of simulations (but see below). Three main sets of simulations were carried out, each consisting of ca. 1000 model runs. The first set reproduced uniform conduit distributions, with model γ_2 values in the range 0.4–0.6; the second set reproduced approximately random conduit distributions ($0.8 < \gamma_2 < 1.2$); and the third set reproduced aggregated distributions ($2.0 < \gamma_2 < 3.0$). The other parameters were identical across the three simulation sets to avoid unwanted variation and were kept constant among simulations except for two parameters: (1) the proportion of the cross-section nodes occupied by conduits, a measure of conduit density, which was varied randomly between 0.1 and 0.5; and (2) Persistence, which was varied randomly between 0 and 1 (i.e., its whole range) to account for the uncertainty in the value of this parameter in the xylem of real plants. Loepefe et al. (2007) conducted extensive simulation experiments across the parameter space of the model (varying average conduit diameter, average conduit length, average maximum pit pore size and the fraction of contact area occupied by pit pores, cf. Table 1), and showed that the effects of connectivity on xylem hydraulic conductivity and vulnerability to embolism were consistent across parameter space.

At low conduit densities and particularly for uniform conduit distributions, it was frequent that the xylem did not completely embolize due to the presence of disconnected clusters of conduits. We only present here the results of the simulations that resulted in complete xylem embolization, and thus a P_{50} value could be properly estimated, but the results remain qualitatively identical if all simulations are considered. We carried out additional sets of simulations using the same parameter values as before (cf. Table 1) and either (1) increasing segment length from 0.2 to 0.5 m, (2) increasing cross-sectional area from 1×10^{-5} to 1×10^{-4} m², or (3) forcing a power relationship between conduit diameter and

length. In all cases, the results were consistent with the initial simulations presented here and are not shown.

RESULTS

As expected, species with different wood type tended to differ in their xylem anatomy (Table 2). Conifer species had more uniform conduit distributions than any other wood type, whereas semi-ring-porous species showed intermediate patterns, and diffuse- and ring-porous species showed similar and more aggregated distributions (Table 2). When all species were pooled together, the slope for the regression between log(conduit density) and log(mean lumen area) (Fig. 1) had a value of -1.00 ± 0.06 (mean \pm SE) according to OLS and -1.20 ± 0.07 according to the MA estimation. The OLS model improved significantly in terms of AIC if the full phylogenetic signal was included using PGLS. Overall, the best model included a categorical variable coding for wood type (conifer, diffuse-porous, semi-ring-porous, ring-porous) and the aggregation index γ_2 (Table 3). The effect of the aggregation index indicated that species with uniform distributions (i.e., low γ_2 , regardless of wood type) had a higher conduit density for a given mean lumen area than species with grouped conduits. Including interaction terms in the model (e.g., allowing the effect of lumen area to vary with γ_2 or different slopes among wood types) did not result in better models in terms of AIC. Similar results were obtained if a different measure of conduit spatial arrangement, based on the distance to the nearest neighbor (cf. Mencuccini et al., 2010), was used in the models instead of γ_2 (not shown).

In addition, we found significant effects of the aggregation index (γ_2) on the conduit lumen fraction (F) and the size to number ratio (S) (Fig. 2), whereas estimated specific hydraulic conductivity (K) was unrelated to γ_2 ($P > 0.2$). Species with

TABLE 1. Values of model parameters and constants used in the simulations. Parameters in boldface were varied in the main simulation experiments reported in the paper.

Symbol	Description	Value
Dimensions of simulated segment		
A_s	Cross-sectional area of the wood segment (mm ²)	10
L_s	Length of the wood segment (mm)	200
L_{ce}	Length of a conduit element (or tracheid) (mm)	2
K	Percentage occupation of cross-section nodes by conduits (determines conduit density)	0.1–0.5
Grouping of conduits		
R	Interaction radius (in units of distance between adjacent nodes)	2
S	Saturation threshold (number of conduits)	3
γ_2	Grouping base (<1: uniform; >1: aggregated)	0.4–0.6; 0.8–1; 2–3
Π	Persistence (probability to start a new conduit; see The Hydraulic Model section in the Materials and Methods)	0–1
Conduits		
D_c	Average conduit diameter (μ m)	34
$D_{c,cv}$	Coefficient of variation of conduit diameters	0.38
P_e	Probability of a node to be a conduit end point	0.97
Interconduit connections		
D_p	Average maximum pit pore size (nm)	50
$D_{p,cv}$	Coefficient of variation of maximum pit pore size	0.5
f_p	Fraction of the contact area occupied by pores	0.35
Propagation of embolism		
N_{focus}	Number of randomly embolized conduits	300
General constants		
γ	Surface tension of water at 25°C (Pa)	$0.072 \cdot 10^{-3}$
μ	Viscosity of water at 25°C (Pa·s)	$1.002 \cdot 10^{-3}$

Notes: Sources of estimated parameter values are Mencuccini et al. (2010) for conduit grouping parameters and Loepefe et al. (2007) for the rest. See Appendix S2 for a detailed description of the model.

TABLE 2. Mean values (\pm SE) and medians (in parentheses) of conduit density, lumen area, and the aggregation index (γ_2) as a function of wood type.

Wood type (No. of species)	Conduit density (mm ⁻²), (median) ^a	Lumen area (μ m ⁻²), (median) ^b	Aggregation index (γ_2), (median) ^c
Conifer (<i>N</i> = 12)	1233 \pm 223, (920) ^A	465 \pm 88, (426) ^A	0.35 \pm 0.04, (0.35) ^A
Diffuse-porous (<i>N</i> = 19)	91 \pm 11, (85) ^B	1529 \pm 211, (1410) ^B	1.72 \pm 0.34, (0.84) ^C
Semi-ring-porous (<i>N</i> = 57)	185 \pm 24, (140) ^C	1495 \pm 129, (1160) ^B	0.68 \pm 0.05, (0.58) ^B
Ring-porous (<i>N</i> = 9)	83 \pm 18, (68) ^B	3118 \pm 818, (2100) ^C	1.21 \pm 0.20, (1.42) ^C

Notes: Different uppercase letters after the median indicate significant differences of the log-transformed values at $\alpha = 0.1$.
^a $F_{3,93} = 54.55$, $P < 0.001$
^b $F_{3,93} = 14.79$, $P < 0.001$
^c $F_{3,93} = 17.24$, $P < 0.001$

more aggregated conduits tended to have lower F (Fig. 2A) and higher S (Fig. 2B). These two relationships remained highly significant and with a similar scaling exponent (within 0.03 of the values shown in Fig. 2) if the extreme value with very low γ_2 at the left of the plots was removed from the analyses. For the two relationships, the model fit improved significantly if phylogenetic structure was taken into account using PGLS and if the effect of wood type was taken into account (Table 4). The effect of γ_2 on F remained negative and highly significant, albeit with a shallower slope, in all models (Table 4). However, the effect of γ_2 on S disappeared when the effects of phylogeny and wood type were accounted for (Table 4).

Regarding model simulations, our results showed that the aggregation coefficient γ_2 affected the topology of the xylem network in 3D, so that xylem with aggregated conduit distributions (in 2D) had a greater number of connected neighbors (i.e., higher conduit-to-conduit connectivity). This effect was apparent throughout the studied range of conduit densities, but was particularly marked at low conduit densities, where the connectivity of aggregated systems almost doubled that of uniform configurations (Fig. 3). Maximum hydraulic conductivity and vulnerability to xylem embolism were also affected by conduit

density and conduit aggregation. As expected, xylem with more conduits had higher conductivities and were more vulnerable to embolism (Fig. 4). For a given conduit density, xylem with aggregated conduits tended to have higher (maximum) hydraulic conductivity (Fig. 4A) and higher vulnerability to xylem embolism (Fig. 4B). This latter effect was particularly noticeable at relatively low conduit densities, as systems with very high conduit densities were always highly vulnerable to embolism (Fig. 4B). According to Loepfe et al. (2007), all the previous effects are predicted to be consistent across parameter space for those parameters that were not varied in the simulations reported here (cf. Materials and Methods section above). Note that xylem with low conduit densities and uniform distributions were not possible in our simulations (cf. Fig. 4) because they led to disjointed clusters of conduits that did not connect through our virtual wood segments (i.e., the hydraulic resistance between the two ends of the segments was infinite).

DISCUSSION

In agreement with our initial hypothesis, the spatial arrangement of xylem conduits was related to other key xylem structural properties, including the conduit packing function, the lumen fraction (F), and the size to number ratio (S). These two latter variables have been proposed as orthogonal axes to characterize the functional implications of conduit packing (Zanne et al., 2010). Differences in conduit aggregation resulted in a significant shift in the intercept of the log-log relationship between lumen area and conduit density, so that woods with an aggregated conduit distribution had a lower conduit density for a given average lumen area. Remarkably, the effect of conduit spatial arrangement remained highly significant, even after accounting for phylogenetic structure and the effect of wood type (cf. McCulloh et al., 2010), and implied a >2-fold difference in the predicted conduit density for a given lumen area for the range of conduit spatial distributions in our data set ($\gamma_2 = 0.1$ –4.8; Table 3 and Appendix S1). It should be noted that the total effect of conduit spatial distribution is possibly larger because wood type and conduit spatial distribution are associated (Table 2), and those wood types with more aggregated distributions had a lower conduit density (everything else being equal, cf. Table 3). Unfortunately, our design does not allow us to completely disentangle these two effects.

This is one of the first studies showing how tissue-level properties and, in particular, the spatial arrangement of conduits in the xylem network, can affect fundamental aspects of xylem function that have traditionally been explored solely from the point of view of the properties of individual conduits and the pits interconnecting them (cf. Carlquist, 1984; Schenk et al., 2008;

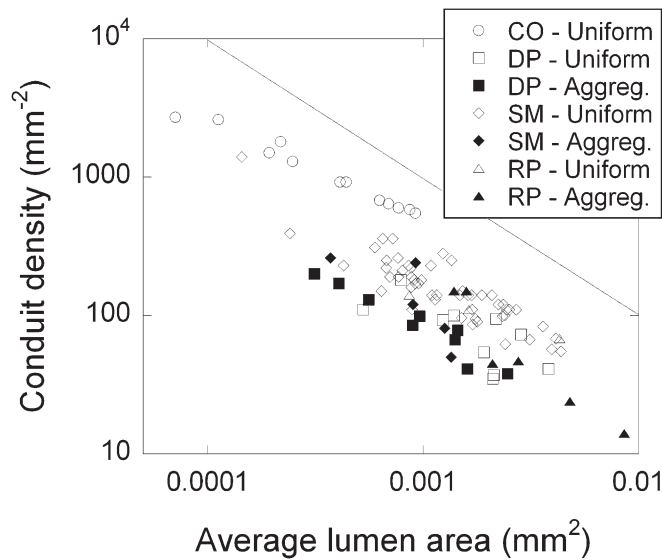


Fig. 1. Relationship between conduit density and average lumen area (packing function) as a function of wood type (CO: conifer, DF: diffuse-porous, SM: semi-ring-porous, RP: ring-porous) and conduit spatial distribution. Conduit spatial distributions were considered aggregate (aggreg.) if $\gamma_2 > 1$ and uniform if $\gamma_2 < 1$ (see text). The fine continuous line indicates the packing limit.

TABLE 3. Summary of models of log(conduit density) as a function of log(average lumen area). Each column corresponds to a different model; cells with a dash indicate explanatory variables not included in a given model. The values are model coefficients (\pm SE), except in the last two rows, corresponding to model degrees of freedom (df) and Akaike information criterion (AIC). The best model includes the phylogenetic signal and the effects of wood type (conifer, diffuse-porous, semi-ring-porous, ring-porous) and the uniformity index γ_2 (last column). Conifers are the reference level in the models accounting for wood type.

Variable	No phylogenetic effects	Models including phylogenetic effects		
Intercept	-1.74 (\pm 0.43) ***	-0.06 (\pm 0.50) ns	1.09 (\pm 0.40) **	0.96 (\pm 0.37) *
Diffuse-porous	—	—	-1.63 (\pm 0.15) ***	-1.42 (\pm 0.14) ***
Semi-ring-porous	—	—	-1.03 (\pm 0.13) ***	-0.95 (\pm 0.11) ***
Ring-porous	—	—	-1.37 (\pm 0.18) ***	-1.20 (\pm 0.17) ***
log(lumen area)	-1.00 (\pm 0.06) ***	-0.77 (\pm 0.06) ***	-0.74 (\pm 0.05) ***	-0.76 (\pm 0.05) ***
γ_2	—	—	—	-0.15 (\pm 0.04) ***
df	97	97	92	91
AIC	159.56	105.67	76.62	71.97

Notes: ns: not significant; *: $P < 0.05$; **: $P < 0.01$; *** $P < 0.001$

and Lens et al., 2011 for some of the very few exceptions). The effect of conduit aggregation on the packing function shows that species with aggregated xylem conduits require fewer conduits

(for a given average lumen area) and a lower conduit lumen fraction (F) to function than do species with uniform conduit distributions. Contrary to the consistency of the effects of conduit aggregation on the packing function and F , the relationship between conduit spatial distribution and the size to number ratio (S) disappeared when the phylogenetic structure of the data were accounted for. This result indicates that this latter relationship has arisen from divergences between major clades, in our case mostly conifers vs. angiosperms (cf. Table 4), rather than arising repeatedly within different clades over evolutionary history.

The effects of conduit spatial distribution outlined in the previous paragraphs support the notion that conduit aggregation leads to more efficient xylem networks with greater water transport capacity. This is consistent with theoretical predictions (Loepfe et al., 2007) and was confirmed by our modeling results. Our simulations show that this effect can be quantitatively important, although a precise evaluation is difficult here because its magnitude (both absolute and relative to the effect of other xylem properties) depends on the exact parameterization of the model. At an intermediate conduit density of 600 mm⁻², our model predicted a 74% increase in connectivity in aggregated vs. uniform xylem networks (Fig. 3), which resulted in a 37% increase in specific hydraulic conductivity (Fig. 4A). If conduit aggregation has such an obvious advantage in terms of water transport, why is it that many species show nearly random or uniform distributions? Our water transport model suggests that hydraulic safety considerations related to the avoidance of drought-induced embolism may be important. Everything else being equal, more aggregated xylem networks tend to favor embolism spread and result in more vulnerable systems (Fig. 4B), in agreement with previous modeling results (Loepfe et al., 2007). Comparable conclusions were reached by Ewers et al. (2007) using a probabilistic approach to model the impact of vessel redundancy on xylem safety, although their model did not explicitly consider connections between vessels.

Interestingly, the species with aggregated conduits in our data set did not realize their potential advantage in terms of hydraulic conductivity. Instead, they had similar hydraulic conductivity but with lower conduit density (for a given average lumen area) and conduit lumen fraction than species with uniform conduit distributions. Fewer conduits imply lower carbon costs of the hydraulic system (sensu McCulloh et al., 2004; Mencuccini et al., 2007) and, everything else being equal, may also provide an advantage in terms of higher resistance to xylem embolism (cf. Fig. 4B). In addition, lower F implies more

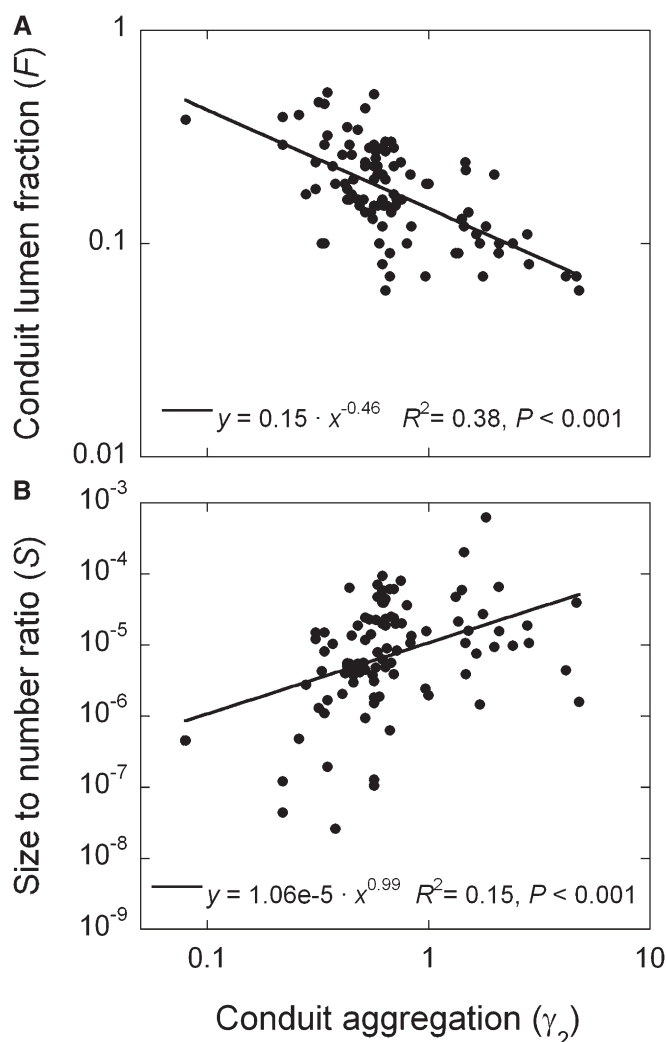


Fig. 2. Relationship between conduit aggregation (parameter γ_2 , cf. Mencuccini et al., 2010) and (A) conduit lumen fraction (F) and (B) conduit size to number ratio (S). Power fits on raw data are shown in each case together with the corresponding model equations. Note the logarithmic scale of all axes.

TABLE 4. Summary of the models of the conduit lumen fraction (F) and the size to number ratio (S) as a function of wood type (conifer, diffuse-porous, semi-ring-porous, ring-porous) and the aggregation index γ_2 . Each column corresponds to a different model; cells with a dash indicate explanatory variables not included in a given model. The values are model coefficients (\pm SE), except in the two last rows, corresponding to model degrees of freedom (df) and Akaike information criterion (AIC). Conifers are the reference level in the models accounting for wood type.

Variable	No phylogenetic effects	Models including phylogenetic effects	
A) Response variable: log <i>F</i>			
Intercept	−1.92 (± 0.05) ***	−1.89 (± 0.09) ***	−1.31 (± 0.15) ***
Diffuse-porous	—	—	−0.94 (± 0.18) ***
Semi-ring-porous	—	—	−0.49 (± 0.15) **
Ring-porous	—	—	−0.54 (± 0.20) **
log γ_2	−0.46 (± 0.06) ***	−0.36 (± 0.07) ***	−0.25 (± 0.07) ***
df	97	97	92
AIC	113.58	96.78	88.68
B) Response variable: log <i>S</i>			
Intercept	−11.45 (± 0.19) ***	−13.30 (± 1.60) ***	−15.22 (± 1.00) ***
Diffuse-porous	—	—	3.69 (± 1.10) **
Semi-ring-porous	—	—	3.41 (± 1.05) **
Ring-porous	—	—	4.62 (± 1.16) ***
log γ_2	1.00 (± 0.24) ***	0.15 (± 0.23) ^{ns}	−0.00 (± 0.25) ^{ns}
df	97	97	92
AIC	376.77	318.93	311.35

Notes: *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.001$

space in the xylem for the storage of water and other resources (Zanne et al., 2010), increased mechanical strength (Baas et al., 2004; see also Zanne et al., 2010) and higher wood density. This latter effect, however, is likely to be quantitatively small, at least in angiosperms because wood density is mostly determined by the density of nonlumen tissue (Zanne et al., 2010).

Another important aspect, highlighted by our modeling results, is the fact that a minimum conduit density is required to have a continuous path of conduits connecting through a given wood segment. Below this minimum density, the two ends of the segment are hydraulically disconnected and water transport

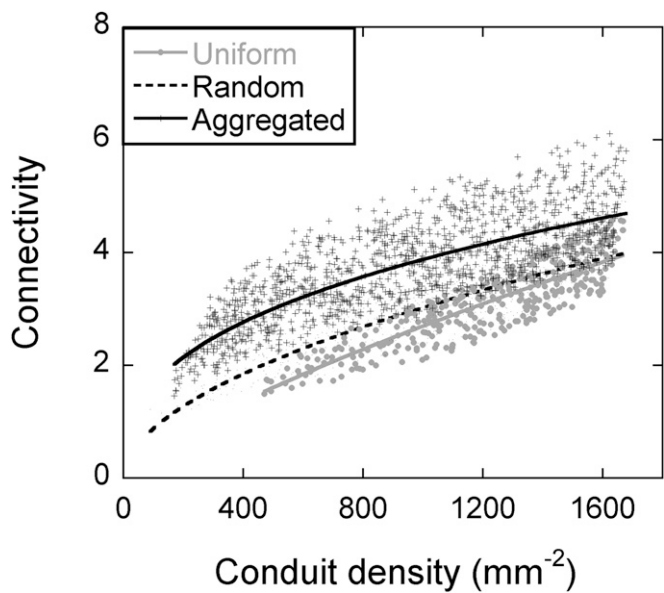


Fig. 3. Relationship between conduit density and conduit-to-conduit connectivity in simulations differing in conduit spatial arrangement. Power fits ($P < 0.001$ in all cases) are shown for each aggregation type. Data points corresponding to random configurations are shown as small dots to improve clarity.

cannot occur (Fig. 4A). What is particularly interesting here is that this minimum conduit density differs depending on the spatial arrangement of the conduits: uniform distributions require a higher minimum density, due to their lower overall connectivity (Fig. 3). This may contribute to explain why species with uniform conduit distributions tend to have higher conduit densities (Fig. 1, Table 3). A limitation of our analysis, however, is that in angiosperms vessels are not the only tracheary cells that are capable of water transport. Some imperforate tracheary elements (unicellular tracheary cells that lack a perforation plate) conduct water; therefore, they are nonnegligible constituents of the xylem network (Sano et al., 2011). In fact, the presence of imperforate tracheary elements has been associated with the spatial distribution of vessels in angiosperms (Carlquist, 1984, 2009), and the role of these nonvessel tracheary cells in the connectivity and overall hydraulic properties of the xylem network should be addressed in future studies.

We would like to stress that our modeling results should be considered as a proof of concept exercise, showing that conduit aggregation per se affects xylem function, rather than an exploration of the trait correlations that are likely to be observed across species in real plants. This is because, in a model such as ours, the effects of individual variables can be isolated by keeping all other variables constant, whereas in real plants variables with opposing effects frequently covary. A case in point is the recent empirical study by Lens et al. (2011), in which it was found that species with a higher vessel grouping index (GI), a measure of conduit aggregation in cross sections, were less conductive and more resistant to embolism (see also Carlquist, 1984, 2009). This is in disagreement with our modeling results, but Lens et al. (2011) also found that hydraulic conductivity on a xylem area basis and vulnerability to embolism increase with vessel length. Vessel length has the effect of increasing the connectivity of the xylem network, thereby increasing hydraulic conductivity and vulnerability to embolism, as correctly predicted by our model (Loepfe et al., 2007). Since vessel length was strongly (and negatively) related to GI in the Lens et al. (2011) data set, it is impossible to infer from their data the net effect of those variables (or of conduit-to-conduit connectivity)

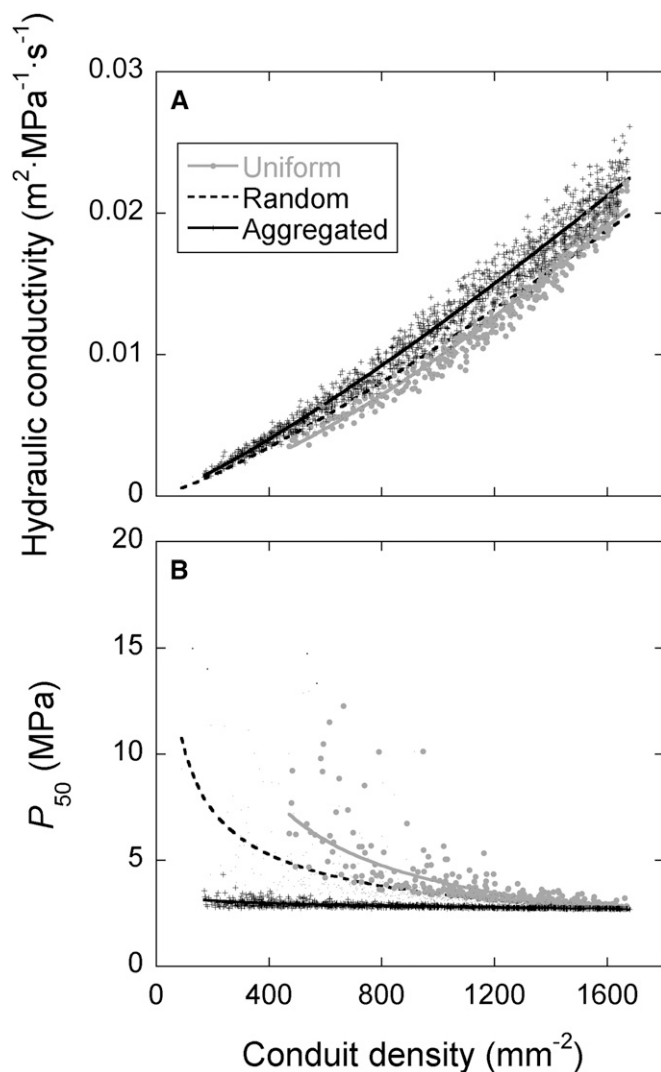


Fig. 4. Relationship between conduit density and (A) specific hydraulic conductivity and (B) vulnerability to xylem embolism (P_{50}) in simulated woods with contrasted conduit aggregation. Power fits ($P < 0.001$ in all cases) are shown for each aggregation type. Data points corresponding to random configurations are shown as small dots to improve clarity of the figure.

on the hydraulic properties of the xylem network. In the modeling results reported here, we chose to keep vessel length distributions constant across simulations to isolate the effects of the spatial arrangement of conduits in cross sections.

A precise determination of the relative importance of conduit spatial arrangement compared to conduit- or pit-level properties in real plants would require empirical measurements of all the key anatomical traits (conduit spatial distribution, conduit density and size distribution, pit properties) and xylem functional properties (hydraulic conductivity, vulnerability to embolism) on the same samples (cf., Lens et al., 2011). Such a data set could be then used to provide a realistic and complete parameterization (and validation) of a model simulating water transport in 3D xylem structures, such as the one presented here.

In conclusion, our study shows that the spatial arrangement of conduits affects the fundamental structural and functional

attributes of the xylem. We believe that both an empirical approach based on extensive measuring of plant traits and their covariation in vivo and a more theoretical approach aimed at disentangling the direct effects of individual traits are required if we are to fully understand the relationship between xylem structure and function and, more generally, the compromises and trade-offs involved in plant drought resistance and its variation within and across species. In that respect, the rigorous analysis of conduit spatial distribution developed by Mencuccini et al. (2010) and expanded here offers a way forward. In the future, empirical and comparative work on the covariation and ecological correlates of the different components of xylem connectivity should be combined with modeling efforts capturing the tradeoffs between these components. Additionally, the analysis of xylem network connectivity should be expanded to the whole tree, where aspects related to the tapering of xylem conduits (Mencuccini et al., 2007; Sperry et al., 2008; McCulloh et al., 2010) and hydraulic integration across stems (Schenk et al., 2008) are likely to become relevant.

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